Supplementary Material

**Shortcomings of the commercial MALDI-TOF MS database and use of MLSA as an arbiter in the identification of *Nocardia* species**

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**Supplementary Table 2│**MLSA identification of isolates with discrepant 16S rRNA and MALDI-TOF MS identifications.

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| --- | --- | --- | --- | --- | --- |
| Isolate | 16S rRNA identification  (percentage similarity with respect to the reference strain) | MALDI-TOF MS identification  (log score) | MLSA identification  (percentage similarity to corresponding GenBank multilocus sequence) | Concordance between 16S rRNA and MLSA identification | Concordance between MALDI-TOF MS and MLSA identification |
| *IDENTIFIED AT GENUS LEVEL* | | | | | |
| HIGH PREVALENCE GROUP | | | | | |
| 20061057 | *N. cyriacigeorgica* (100%) | *N. abscessus*2 (2.104) | *N. kruczakiae* (98.1%) | No | No |
| 20071090 | *N. nova* (99.9%) | *N. aobensis*1 (1.595) | *N. aobensis* (98.9%) | No | Yes |
| 20090138 | *N. nova* (100%) | *N. otitidiscaviarum*4 (1.971) | *N. puris/N. cerradoensis* (95.4%) | No | No |
| 20090226 | *N. nova* (100%) | *N. carnea*1 (1.299) | *N. testacea* (94.4%) | No | No |
| 20100997 | *N. nova* (99.4%) | *N. aobensis*1 (1.587) | *N. puris* (94.9%) | No | No |
| INTERMEDIATE PREVALENCE GROUP | | | | |  |
| 20071291 | *N. brasiliensis* (100%) | *Nocardia* sp. MB9090 0JTHL2 (1.896) | *N. brasiliensis* (96.9%) | Yes | No |
| 20081631 | *N. carnea* (99.6%) | *N. farcinica*1 (1.389) | *N. puris* (97.0%) | No | No |
| 20111159 | *N. brasiliensis* (99.7%) | *Nocardia* sp. MB9090 0JTHL1 (2.200) | *N. brasiliensis* (97.4%) | Yes | No |
| 20070999 | *N. transvalensis* complex (99.8%) | *N. africana*1 (1.298) | *N. wallacei* (97.6%) | No | No |
| LOW PREVALENCE GROUP | | | | |  |
| 20060216 | *Nocardia* sp. T42 (100%)\* | *N. paucivorans*1 (1.756) | *N. paucivorans* (100%) | No | Yes |
| 20070386 | *Nocardia s*p. JCM 3016 (99.8%)\* | *N. nova*2 (2.019) | *N. wallacei* (98.0%) | No | No |
| 20070400 | *N. exalbida* (99.9%) | *N. abscessus*1 (1.601) | *N. arthritidis* (98.8%) | No | No |
| 20071169 | *N. vinacea* (99.8%)\* | *N. aobensis*1 (1.913) | *N. asteroides* (96.9%) | No | No |
| 20071587 | *Nocardia* sp. 171747 (96.7%)\* | *N. farcinica*1 (1.746) | *N. farcinica* (99.0%) | No | Yes |
| 20071746 | *Nocardia* sp. (98.1%)\* | *N. concava*2 (1.376) | *N. concava* (99.3%) | No | Yes |
| 20080233 | *N. wallacei* (99.8%)\* | *N. sienata*1 (1.550) | *N. sienata* (97.0%) | No | Yes |
| 20090880 | *N. neocaledoniensis* (98.6%) | *N. asteroides*4 (1.309) | *N. asteroides* (98.0%) | No | Yes |
| 20091616 | *N. rhamnosiphila* (100%)\* | *N. testacea*1 (1.501) | *N. rhamnosiphila* (100%) | Yes | No |
| 20091663 | *N. testacea* (99.8%) | *N. sienata*1 (1.652) | *N. sienata* (99.1%) | No | Yes |
| 20091803 | *N. cerradoensis* (99.7%) | *N. nova*2 (1.484) | *N. cerradoensis* (99.2%) | Yes | No |
| 20091823 | *N. pneumoniae* (99.3%) | *N. farcinica*3 (1.724) | *N. pneumoniae* (97.7%) | Yes | No |
| 20091843 | *N. rhamnosiphila* (99.9%)\* | *N. sienata*1 (1.498) | *N. testacea* (97.4%) | No | No |
| 20091844 | *Nocardia* sp. FSN35 (99.6%)\* | *N. testacea*1 (1.522) | *N. sienata* (97.0%) | No | No |
| 20100053 | *N. jiangxiensis* (100%)\* | *N. elegans*2 (1.357) | *N. puris* (94.7%) | No | No |
| 20100128 | *N. grenatensis* (99.6%)\* | *Nocardia sp*3*.* (1.366) | *N. rhamnosiphila* (98.7%) | No | No |
| 20101005 | *N. wallacei* (99.8%)\* | *N. testacea*1 (1.623) | *N. rhamnosiphila* (99.8%) | No | No |
| 20101525 | *N. cerradoensis* (99.5%) | *N. aobensis*1 (1.372) | *N. aobensis* (95.1%) | No | Yes |
| 20120097 | *N. shimofusensis* (99.8%)\* | *Nocardia* sp.2 (1.381) | *N. higoensis* (95.7%) | No | No |
| 20120308 | *N. pneumoniae* (99.2%) | *N. aobensis*1 (1.647) | *N. pneumoniae* (96.5%) | Yes | No |
| 20120772 | *N. ignorata* (99.6%) | *N. asteroides*1 (1.382) | *N. pneumoniae* (94.4%) | No | No |
| 20130579 | *Nocardia* sp. FSN35 (99.7%)\* | *N. sienata*1 (1.399) | *N. testacea* (97.5%) | No | No |
| 20130718 | *Nocardia* sp. 84317 (99.5%)\* | *N. asiatica*1 (1.526) | *N. puris* (94.9%) | No | No |
| 20130720 | *Nocardia* sp*.* FSN35 (100%)\* | *N*. *testacea*1 (1.527) | *N. testacea* (97.4%) | No | Yes |
| 20130759 | *N. cerradoensis* (99.8%) | *N. nova*2 (1.725) | *N. cerradoensis* (99.2%) | Yes | No |
| *MISSIDENTIFIED AT BOTH SPECIES AND GENUS LEVEL* | | | | |  |
| HIGH PREVALENCE GROUP | | | | |  |
| 20080801 | *N. farcinica* (99.9%) | *Staphylococcus capitis* ssp. *urealyticus*2 (1.407) | *N. farcinica* (99.8%) | Yes | No |
| INTERMEDIATE PREVALENCE GROUP | | | | |  |
| 20060952 | *N. carnea* (99.9%) | *Rhodococcus rhodochrous*1 (1.287) | *N. carnea* (95.7%) | Yes | No |
| 20070252 | *N. transvalensis* complex (99,7%) | *Weissella viridescens*1 (1,730) | *N. wallacei* (98.2%) | No | No |
| 20070519 | *N. transvalensis* complex (99,7%) | *Lactobacillus plantarum*1 (1,286) | *N. wallacei* (98.3%) | No | No |
| 20080303 | *N. carnea* (99.6%) | *Lactobacillus fructivorans*1 (1.271) | *N. wallacei* (96.4%) | No | No |
| 20100800 | *N. carnea* (99.8%) | *Lactobacillus paralimentarius*3 (1.428) | *N. carnea* (95.0%) | Yes | No |
| 20101207 | *N. carnea* (99.6%) | *Agromyces rhizospore*1 (1.360) | *N. puris* (97.2%) | No | No |
| 20110040 | *N. carnea* (99.7%) | *Lactobacillus fructivorans*1 (1.453) | *N. carnea* (95.6%) | Yes | No |
| LOW PREVALENCE GROUP | | | | |  |
| 20060048 | *N. beijingensis* (99.8%)\* | *Lactobacillus brevis*1 (1.262) | *N. pneumoniae* (96.5%) | No | No |
| 20060423 | *N. ignorata* (99.9%) | *Legionella dumoffii*1 (1.262) | *N. asteroides* (94.2%) | No | No |
| 20060742 | *N. beijingensis* (99.7%)\* | *Candida auris*3 (1.314) | *N. pneumoniae* (96.5%) | No | No |
| 20060907 | *N. wallacei* (99.7%)\* | *Staphyloococcus haemolyticus*1 (1.368) | *N. wallacei* (98.2%) | Yes | No |
| 20070689 | *N. ignorata* (99.6%) | *Lactobacillus amylovorus*4 (1.189) | *N. ignorata* (98.9%) | Yes | No |
| 20080144 | *N. takedensis* (100%)\* | *Candida krusei*1 (1.227) | *N. ignorata* (98.1%) | No | No |
| 20080320 | *Nocardia* sp. PK2002 (99.8%)\* | *Arthrobacter stackebrandtii*1 (1.504) | *N. pneumoniae* (97.4%) | No | No |
| 20080566 | *N. beijingensis* (100%) | *Candida krusei*1 (1.421) | *N. beijingensis* (99.1%) | Yes | No |
| 20080687 | *N. asteroides* (99.3%) | *Klebsiella pneumoniae*1 (1.410) | *N. puris* (95.1%) | No | No |
| 20080895 | *N. flavorosea* (99.3%)\* | *Kocuria* sp*.*1 (1.392) | *N. flavorosea* (95.6%) | Yes | No |
| 20080920 | *N. paucivorans* (99.8%) | *Arthrobacter russicus*1 (1.100) | *N. paucivorans* (95.7%) | Yes | No |
| 20081082 | *N. takedensis* (99.8%)\* | *Candida druse*1 (1.277) | *N. cyriacigeorgica* (97.4%) | No | No |
| 20081312 | *N. ignorata* (99.6%) | *Trichosporon mucoides*1 (1.304) | *N. ignorata* (98.4%) | Yes | No |
| 20081439 | *N. puris* (99.7%)\* | *Clostridium septicum*5 (1.376) | *N. puris* (99.2%) | Yes | No |
| 20081632 | *N. ignorata* (99.6%) | *Salmonella* sp.1 (1.240) | *N. ignorata* (98.9%) | Yes | No |
| 20090011 | *N. asteroides* (99.9%) | *Lactobacillus rhamnosus*1 (1,989) | *N. asteroides* (96.6%) | Yes | No |
| 20090451 | *Nocardia* sp. PK2002 (99.7%) | *Acidovorax temeprans*1 (1.343) | *N. puris* (92.3%) | No | No |
| 20090803 | *N. beijingensis* (100%)\* | *Trichospora mucoides*1 (1.460) | *N. araoensis* (97.3%) | No | No |
| 20090922 | *Nocardia* sp. PK2002 (99.0%) | *Lactobacillus brevis*4 (1.459) | *N. araoensis* (96.3%) | No | No |
| 20091802 | *Nocardia* sp. T42 (99.8%) | *Lactobacillus fructivorans*1 (1.367) | *N. carnea* (95.3%) | No | No |
| 20100098 | *N. beijingensis* (100%)\* | *Lactobacillus fructivorans*1 (1.228) | *N. arthritidis* (97.3%) | No | No |
| 20111234 | *N. niigatensis* (99.9%)\* | *Streptomyces griseus*1 (1.285) | *N. niigatensis* (96.4%) | Yes | No |
| 20120055 | *N. veterana* (99.7%) | *Streptococcus mutans*1 (1.443) | *N. veterana* (99.4%) | Yes | No |
| 20130578 | *N. elegans* (99.7%) | *Cryptococcus uniguttulatus*3 (1.118) | *N. veterana* (99.2%) | No | No |
| 20130836 | *N. ignorata* (99.2%) | *Lactobacillus plantarum*1 (1.383) | *N. ignorata* (98.6%) | Yes | No |

\*: Species not present in the MALDI-TOF MS Bruker commercial database at the time of study

1: Protein extraction viathe Verroken *et al*. method

2: Protein extraction viathe Verroken *et al*. method after mechanical disruption with glass beads

3: Protein extraction viathe Verroken *et al*. method after freezing for 48 h

4: Protein extraction viathe Verroken *et al*. method with 10 min of prior sonication